

DETERMINATION OF LEPTIN RECEPTOR IN THE SERUM AND RELATIONS TO LABORATORY AND ANTHROPOLOGICAL PARAMETERS IN PATIENTS WITH ATHEROSCLEROTIC COMPLICATIONS

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Leptin receptors are supposed to have signal effects and are located in most tissues in the organism but we failed to find literary data on concentration (measurement) of leptin receptors in the system circulation. We examined by the method of randomized selection the group of 20 patients with manifested atherosclerosis in whom BMI was calculated. Then we analyzed concentration of leptin receptor (double sandwich ELISA, standard recombinant human leptin), leptin, glucose, insulin, proinsulin, CRP and uric acid in the serum. The control group consisted of 103 probands without signs of atherosclerosis or other manifested diseases. The control group was subjected to determination of BMI, leptin and leptin receptor in the serum. Concentration of leptin receptor does not differ significantly between the patients with atherosclerosis and normal population. Probands with atherosclerosis showed a very close negative correlation between concentration of leptin receptor and leptinemia which is absent in normal population.

INTRODUCTION

Several years ago, in animal experiments there was localized the obese gene (Ob gene) encoding the protein weighing 16 kD, which consists of 167 amino acids. This protein is called leptin^{1,2}.

Leptin is produced by mature adipocytes into system circulation and its primary effect is expected in the hypothalamus^{1,2,3,4}. Leptin receptors are present in most tissues (chorioid plexus, nucleus arcuatus, ventromedial, paraventricular and dorsomedial thalamus, in eminencia mediana of the hypothalamus, in hippocampus, gyrus dentatus, at low concentration also in the piriform cortex and medial border of the medial habenular nucleus. The receptor for leptin is probably located on neurons containing neuropeptide Y. In addition to CNS, leptin receptor occurs in the lung, liver, heart, renal marrow, beta cells of the pancreas, adrenal marrow, ovaries, intestine mucosa, spleen and primary hematogenous cells).

Localization of leptin receptors predetermines leptin and similar molecules for numerous important organ-specific functions.

We dispose of methods for measurement of concentration of free leptin receptor in the system circulation⁵ but no report on similar methods nor information about receptor concentration in system circulation were avail-

able. Therefore we focussed on relations between leptinemia and concentration of leptin receptor in venous blood in patients with manifested atherosclerosis. Attention was also paid to possible differences in concentrations of leptin receptor in such patients compared to normal population.

EXPERIMENTAL PART

Using the method of randomized selection, we examined the group of 20 probands, patients of the Metabolic and Diabetologic Center at the Hospital Šternberk who were treated there for dyslipidemia. All probands underwent either coronary or cerebral attack (according to their case histories) or showed symptoms of angina pectoris (I–III according to CCS).

The control group consisted of 103 probands who were sent for examination at the Department of Laboratory Medicine, Hospital Šternberk, within the preventive screening program and were not followed up for any complication of manifested atherosclerosis, nor had significant aggregation of risk factors (more than two risk factors).

All the probands were examined for leptin concentration (ELISA, BioVendor) and BMI index was calculated. Patients with dyslipidemia and complications of

atherosclerosis were also examined for serum concentration of insulin (CLIA-IMMULITE, DPC), intact proinsulin (ELISA, DAKO), glucose (ILAB-600, BioVendor), cholesterol (ILAB-600, BioVendor), HDL cholesterol (ILAB-600, BioVendor), triacylglycerols (ILAB-600, BioVendor), uric acid (ILAB-600, BioVendor), and CRP (ILAB-600, BioVendor). In all probands, the atherogenic index of plasma was calculated⁶.

In both the groups we determined concentration of free leptin receptor. The analysis was performed using the set of the company BioVendor (**"Human Leptin receptor ELISA"**). This is a double sandwich method using the pair of mice monoclonal antibodies, one of them being bound on a microtitration plate and the other is conjugated with horseradish peroxidase; TMB is used as a substrate. The standard is a recombinant leptin receptor (its binding part for leptin which is connected with Fc fragment of human IgG. The measured concentrations in the population range within 40–50 ng/ml according to our published results⁵. This set was tested by us in our previous studies and we obtained very good analytical results⁵.

The measured and calculated data were processed by the statistical program STATGRAFICnew 5.

RESULTS

We examined the group of twenty persons with complications of manifested atherosclerosis (9 men, 11 women) who were treated at the Metabolic Center. All probands were followed up for hypertension (therapy with selective beta blockers, calcium blockers, ACE-inhibitors and diuretics), dyslipidemia (therapy with statins, fibrates). Five probands (25%) of this group were followed up also for impaired glucose tolerance; none of the probands had diabetes mellitus. None of the probands was an active smoker nor reported a higher daily consumption of coffee (over one cup daily) or alcohol (over 15 g daily) (Table 1).

Table 1. Anamnesis in group with atherosclerosis (N = 20)

Characteristics	Abs.	Percent
Impaired glucose tolerance	5	25%
Nicotinism	0	0%
Ethylic	0	0%
Hypertension	20	100%
Hypolipidemic therapy-statins	19	95%
Hypolipidemic therapy-fibrates	8	40%
MI or unstable AP	17	83%
Stroke	3	17%

With regard to biochemical and anthropological point of view, the group under study can be characterized as persons without signs of obesity or lipidemia, with normal glycemia concentration, insignificant hyperleptinemia, and with insignificantly increased atherogenic plasma index. This group can be classified as correctly treated persons (Table 2).

Table 2. Patients with atherosclerosis (N = 20).

Parameter	X	S	LQ	UQ	Min	Max	T-score
Age	63.3	21.2	52	78	24	91	xxx
BMI	27.9	4.5	25.7	29.7	21.1	35.7	1.95
Insulin	6.8	1.9	6	7	5	10	-0.04
Proinsulin	2.8	1.3	2	3	2	5	0.57
Leptin	17.3	17.6	4.7	19.9	2.6	57	2.06
LP/BMI	0.58	0.51	0.18	0.7	0.1	1.6	xxx
LR	138.8	57.3	88	186	44	220	xxx
LP/LR	0.24	0.37	0.02	0.26	0.01	1.3	xxx
Glucose	5.1	2.9	4.2	5.3	3.8	6.8	0.4
Cholesterol	4.8	1.3	4	6	3	6	0
HDL	1	0.3	0.9	1.1	0.8	1.4	0
Triglycerides	1	0.5	0.9	1.4	0.8	2.1	0
LDL	3.3	1.7	2	4.5	1	5	1
Urates	268.8	76.2	213	325	196	371	-0.8
AIP	0.11	0.24	-0.05	0.2	-0.5	0.6	1.2
CRP	6.8	4.3	4	9.5	3	13	0.6

X = mean, S = standard deviation, lower quartile, UQ = upper quartile, Min = minimum, Max = maximum, AIP = plasma atherogenic index

The group of 20 probands with manifested atherosclerosis was studied for possible correlation of the above mentioned parameters. We found correlations between leptinemia and BMI value, and between uricemia and proinsulin concentration. We also identified correlation between uricemia and CRP concentration in the serum, as well as a significant, very close negative correlation between concentration of leptin receptor (LR) and leptinemia and BMI value. Concentration of leptin receptor correlated negatively with leptinemia related to BMI (LP/BMI). On the contrary, BMI correlated positively with leptinemia related to concentration of leptin receptor (LP/LR) (Table 3, graph No. 1).

Table 3. Correlation in patients with atherosclerosis (N = 20) and in control group (N = 103).

Parameter	Atherosclerosis	Control group	
	VS	VS	NS
LR	leptin(-0.84)		leptin (-0.29)
LR	BMI (-0.74)		BMI (-0.21)
LP/BMI	LR (-0.81)	LR (-0.8)	
LP/LR	BMI (0.7)	BMI (0.75)	
Proinsulin	urates(0.6)	no available	
CRP	urates(0.65)	no available	
Leptin	BMI (0.67)	BMI (0.7)	

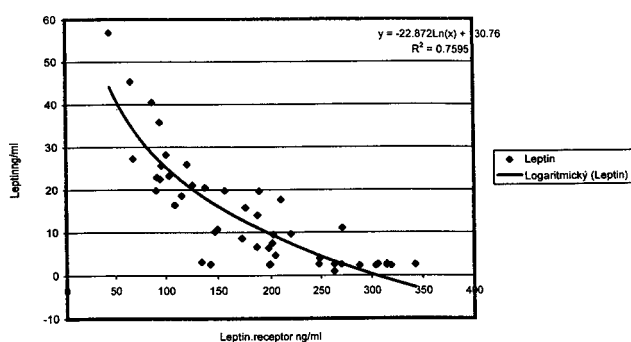
VS....very significant, 99%

() correlation coef

S....significant, 95%

NS....no significant

Graph 1. Leptin and leptin receptor in patients with atherosclerosis



Then we performed basic examination of the control group (103 probands, 64 women, 39 men). These probands were examined within the preventive screening program and had not been followed up nor treated for complications of atherosclerosis, diabetes mellitus or hypertension. None of them was an active smoker nor reported a significantly higher intake of alcohol (over 15 g daily) or coffee (over 1 cup daily). From the anthropological point of view, this group can be characterized as persons without obesity signs. The age of control probands did not differ significantly from that of probands with manifested atherosclerosis. Similarly, the values of leptin and leptin receptor did not differ significantly in both the groups (Table 4).

Table 4. Control group (N = 101).

Parameter	X	S	Median	LQ	UQ	RR
LR	153.2	94.1	130	102	172	NS
Leptin	14	13.2	11	2	20	NS
LP/BMI	0.67	0.38	0.6	0.4	0.8	NS
LP/LR	0.16	0.17	0.16	0.1	0.4	NS
Age	55	20.3	47	59	71	NS
BMI	27.7	3.9	28	25	30	NS

X = mean, S = standard deviation, lower quartile, UQ = upper quartile, Min = minimum, Max = maximum, AIP = plasma atherogenic index, RR = difference between groups

The control group was examined for possible correlations of the mentioned parameters. We found a significant close correlation between leptinemia and BMI value, between LP/LR index and BMI, and a negative correlation between leptin receptor and LP/BMI index. No correlation was found between concentration of leptin receptor in the serum and leptinemia or BMI in the group of "healthy" persons (Table 3).

DISCUSSION

Leptin in the organism is circulating being bound on binding protein which affects its metabolic clearance, biological availability and effectiveness. Binding proteins provide, among others, bonds on leptin receptors and transfer via HEB (one hypothesis supposes that the circulating leptin can be bound on a soluble receptor for leptin that allows an easy transfer of leptin via HEB⁴). Obese persons have higher values of free leptin. Only free leptin is biologically active⁴. Non-obese subjects have leptin mostly bound on the binding substance⁴, which eliminates the inhibitory effect of leptin on appetite. On the contrary, obese individuals show to have most leptin in a free form that is biologically effective. Unfortunately, impaired function of leptin receptor in human accounts for failure of biological effectiveness of leptin⁴.

Impaired function of leptin receptor is a typical example of genetic model of obesity. Obesity in human is associated with leptin resistance¹. Another mechanism of leptin resistance (besides disorders of the receptor and postreceptor function) and subsequently cause of obesity can be saturation of leptin carrier via HEB.

It is supposed that a short isoform of leptin receptor (the existence of one long isoform and at least four short isoforms was proved) the concentration of which was determined in the serum, is responsible for leptin transport and transmission of "leptin" information, thus playing a key role in clearance of leptin. However, it is not clear yet whether the short form is fully biologically active (in contrast to the long isoform) because it has no signal activity.

Expression of leptin receptors is changed, similarly as leptin expression (probably this is the true reason) by numerous hormones and elements. We failed to find any paper reporting about methods of measurement of leptin receptor in the serum so that we have no information about any modification of leptin receptor in the system circulation (although several factors can exist). If we suppose certain analogy with other systems in the organism, concentration of leptin receptor should be lower in obese individuals with hyperleptinemia.

We think that knowledge of leptin receptor concentration, particularly knowledge of relations among numerous syndromes and symptoms of "civilization diseases" and the leptin receptor could elucidate many problems.

Therefore interesting enough is the finding of an important correlation between the concentration of leptin and leptin receptor in patients with manifested complications of atherosclerosis. This correlation is expressed even at calculation of leptin to BMI index (LP/BMI) which should eliminate partially possible interference associated with leptin receptor concentration and BMI. However, such a correlation was found only in patients with manifested complications of atherosclerosis. Individuals without these symptoms or diseases showed no correlation between leptinemia and leptin receptor concentration (we found only a negative corre-

lation between leptinemia related to BMI and leptin receptor concentration).

Our analyses suggest that correlation between leptinemia and leptin receptor concentration in the serum is expressed significantly in patients with manifested atherosclerosis and normal leptin levels in the serum; such a correlation could not be causal in normal population but exists only in relation to adipose tissue (BMI).

It can indicate that in patients with manifested atherosclerosis (endothelial dysfunction), when leptin production is influenced by many factors that are in causal association with atherosclerosis (and adipose tissue could not be the main and only determinant of leptin synthesis), leptin production can be “partly autonomous”, independent of quantity of adipose tissue. In such a case, regulation of leptin effect could be affected directly by the negative feedback (production of the receptor for leptin – if the receptor is considered as an effector – carrier of information).

In healthy probands, leptin production is constant with respect to quantity and types of adipose tissue that is the major determinant of leptinemia. A simple correlation between leptinemia and concentration of leptin receptor may not be expressed so explicitly.

Another, still unknown effect of leptin (its receptor) in the process of atherogenesis cannot be excluded.

CONCLUSION

We examined concentration of leptin receptor (ELISA) in venous blood in the group of 20 patients with complications of atherosclerosis and also in the group of 103 probands without symptoms of atherosclerotic complications or other severe diseases. It was found that in patients with atherosclerosis the concentration of leptin receptor was in a very close negative correlation with leptinemia (even with leptinemia related to BMI). The group of “healthy” probands showed a less close correlation (leptinemia should be related to BMI).

Concentration of leptin receptor and/or its relation to leptin or BMI did not differ significantly in both groups.

Our findings could suggest that leptin production in patients with atherosclerosis is not dependent only on quantity and type of adipose tissue, but depends on many others factors (endothelial dysfunction?) and is regulated directly by production of leptin receptor (which probably has an effector function). It cannot be excluded (several hypotheses appeared) that leptin and its receptor display a still unknown effect in the process of atherogenesis.

In persons of the same physical constitution without atherosclerosis, leptin receptor may play an important role in regulation of leptin effect, but these relations are not so close and decisive can be the adipose tissue.

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